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TRANSMITTAL LETTER TO THE UNITED STATES

ATTORNEY'S DOCKET NUMBER 0050/50669

DESIGNATED/ELECTED OFFICE (DO/E.O./US)
CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

INTERNATIONAL APPLICATION NO.
PCT/EP 00/08222

INTERNATIONAL FILING DATE
August 23, 2000

PRIORITY DATE CLAIMED
September 1, 1999

TITLE OF INVENTION: FATTY ACID DESATURASE GENE FROM PLANTS

APPLICANT(S) FOR DO/E.O./US Ivo FEUSSNER; Ellen HORNING; Kathrin FRITSCH; Nicola PEITZSCH and Andreas RENZ

Applicant herewith submits to the United States Designated/Elected Office (DO/E.O./US) the following items and other information:

1. /X/ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
 2. / / This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
 3. /X/ This express request to begin national examination procedures (35 U.S.C.371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
 4. / / A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
 5. /X/ A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a./X/ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b./ / has been transmitted by the International Bureau.
 - c./ / is not required, as the application was filed in the United States Receiving Office (RO/USO).
 6. /X/ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
 7. /X/ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
 - a./X / are transmitted herewith (required only if not transmitted by the International Bureau).
 - b./ / have been transmitted by the International Bureau.
 - c./ / have not been made; however, the time limit for making such amendments has NOT expired.
 - d./ / have not been made and will not be made.
 8. /X/ A translation of the amendments to the claims under PCT Article 19(35 U.S.C. 371(c)(3)).
 9. /X/ An oath or declaration of the inventor(s)(35 U.S.C. 171(c)(4)).
 10. / / A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).
- Items 11. to 16. below concern other document(s) or information included:
11. / / An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
 12. /X/ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
 13. /X/ A FIRST preliminary amendment.
/ / A SECOND or SUBSEQUENT preliminary amendment.
 14. / / A substitute specification.
 15. / / A change of power of attorney and/or address letter.
 16. /X/ Other items or information.
International Search Report
International Preliminary Examination Report

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10/069772

U.S. Appln. No. (If Known) INTERNATIONAL APPLN. NO. ATTORNEY'S DOCKET NO.
PCT/EP 00/08222 0050/50669

	CALCULATIONS	PTO USE ONLY
17. /X/ The following fees are submitted		
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):		
Search Report has been prepared by the		
EPO or JPO.....	\$890.00 890.00	
International preliminary examination fee paid to USPTO		
(37 CFR 1.482).....	\$710.00	
No international preliminary examination fee paid to		
USPTO (37 CFR 1.482) but international search fee paid		
to USPTO (37 CFR 1.445(a)(2)).....	\$740.00	
Neither international preliminary examination fee		
(37 CFR 1.482) nor international search fee		
(37 CFR 1.445(a)(2)) paid to USPTO	\$1040.00	
International preliminary examination fee paid to		
USPTO (37 CFR 1.482) and all claims satisfied pro		
-visions of PCT Article 33(2)-(4).....	\$100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	\$ 890.00	
Surcharge of \$130.00 for furnishing the oath or declaration		
later than / / 20 / / 30 months from the earliest		
claimed priority date (37 CFR 1.492(e)).		
Claims	Number Filed	Number Extra
Total Claims	-20	
Indep. Claims	-3	
Multiple dependent claim(s) (if applicable)		
		Rate
		X\$18.
		X\$84.
		+280.
TOTAL OF ABOVE CALCULATION		=
Reduction of 1/2 for filing by small entity, if applicable.		
Verified Small Entity statement must also be filed		
(Note 37 CFR 1.9, 1.27, 1.28).		
SUBTOTAL		= 890.00
Processing fee of \$130. for furnishing the English		
translation later than / / 20 / / 30 months from the		
earliest claimed priority date (37 CFR 1.492(f)). +		
TOTAL NATIONAL FEE		= 890.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)).		
The assignment must be accompanied by an appropriate cover		
sheet (37 CFR 3.28, 3.31) \$40.00 per property		= 40.00
TOTAL FEES ENCLOSED		= \$ 930.00
Amount to be		
refunded:	\$	
Charged	\$	

a./X/ A check in the amount of \$930.00 to cover the above fees is enclosed.

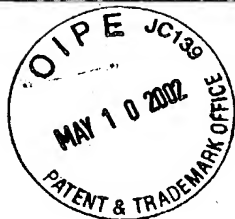
b./ / Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c./X/ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 11-0345. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
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1101 Connecticut Ave., N.W.
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SIGNATURE
Herbert B. Keil
NAME
18,967
Registration No.



1.0069772.022302
JC02 Rec'd PCT/PTO PO MAY/2002 SBCO
#4

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)

FEUSSNER et al.)

Serial No. 10/069,772)

Filed: February 28, 2002)

For: FATTY ACID DESATURASE GENE FROM PLANTS)

BOX PCT

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on:

Date of Deposit	May 9, 2002
Person Making Deposit	Herbert B. Keil
Signature	Herbert B. Keil
Date of Signature	May 9, 2002

Honorable Commissioner of
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Washington, D.C. 20231

PRELIMINARY AMENDMENT
AND
RESPONSE TO NOTICE TO COMPLY WITH SEQUENCE REQUIREMENTS

Sir:

In response to the Notification to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures, a copy of the Sequence Listing in computer readable form is attached hereto. The content of the paper copy of the Sequence Listing and the copy of the Sequence Listing in computer readable form is the same, and includes no new matter.

AMENDMENTS

IN THE SPECIFICATION:

Delete the sequence listing in the specification and substitute with the attached replacement sequence listing as separate pages 1-4.

REMARKS

It is believed that by submitting the present amendment and sequence listing diskette, the application now fully complies with the requirements of 37 CFR 1.821-1.825. Favorable action by the examiner is solicited.

Please charge any shortage in fees due in connection with the filing of this paper, including Extension of Time fees to Deposit Account No. 11-0345. Please credit any excess fees to such account.

Respectfully submitted,

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10/069772

JC19 Rec'd PCT/PTO 2 8 FEB 2002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

FEUSSNER et al.

PCT/EP 00/08222

Intl. Filing Date: August 23, 2000

US Serial No.: TO BE ASSIGNED

For: FATTY ACID DESATURASE GENE FROM PLANTS

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

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PRELIMINARY AMENDMENT

Sir:

Prior to examination of the above-identified U.S. National
Stage application, kindly amend the application as follows.

CLEAN VERSION OF ALL CLAIMS

1. An isolated nucleic acid sequence which encodes a polypeptide with desaturase activity, selected from the following group:

- a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 1,
- b) nucleic acid sequences which, as a result of the degeneracy of the genetic code, are derived from the nucleic acid sequence shown in SEQ ID NO: 1,
- c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 1 which encode polypeptides with the amino acid sequences shown in SEQ ID NO: 2 and which have at least 75% homology at amino acid level without substantially reducing the enzymatic activity of the polypeptides.

2. An amino acid sequence encoded by a nucleic acid sequence as claimed in claim 1.

3. An amino acid sequence as claimed in claim 2, encoded by the sequence shown in SEQ ID NO: 1.

4. A nucleic acid construct comprising a nucleic acid sequence as claimed in claim 1, where the nucleic acid sequence is linked to one or more regulatory signals.

5. (amended) A vector comprising a nucleic acid sequence as claimed in claim 1 or a nucleic acid construct comprising said

nucleic acid sequence linked to one or more regulatory signals.

6. (amended) An organism comprising at least one nucleic acid sequence as claimed in claim 1 or at least one nucleic acid construct comprising said nucleic acid sequence linked to one or more regulatory signals.

7. An organism as claimed in claim 6, which is a plant, a microorganism or an animal.

8. (amended) A transgenic plant comprising a functional or nonfunctional nucleic acid sequence as claimed in claim 1 or a functional or nonfunctional nucleic acid construct comprising said nucleic acid sequence linked to one or more regulatory signals.

9. (amended) A process for the preparation of unsaturated fatty acids, which comprises introducing at least one nucleic acid sequence as claimed in claim 1 or at least one nucleic acid construct comprising said nucleic acid sequence linked to one or more regulatory signals into an oil-producing organism, growing this organism, isolating the oil contained in the organism and liberating the fatty acids contained in the oil.

10. (amended) A process for the preparation of triglycerides with an increased content of unsaturated fatty acids, which comprises introducing at least one nucleic acid sequence as claimed in claim 1 or at least one nucleic acid construct comprising said nucleic acid sequence linked to one or more regulatory signals into

11. (amended) A process for the preparation of saturated fatty acids, which comprises introducing at least one nonfunctional nucleic acid sequence as claimed in claim 1 or at least one nonfunctional nucleic acid construct comprising said nucleic acid sequence linked to one or more regulatory signals into an oil-producing organism, growing this organism, isolating the oil contained in the organism and liberating the fatty acids contained in the oil.

13. (amended) A process as claimed in claim 9, wherein the unsaturated fatty acids have an increased calendulic acid content.

15. An unsaturated fatty acid prepared by a process as claimed in claim 9.

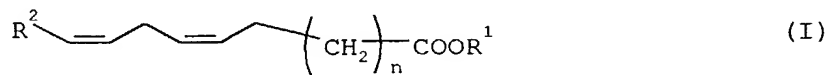
16. A triglyceride with an increased content of unsaturated fatty acids prepared by a process as claimed in claim 10.

17. A saturated fatty acid prepared by a process as claimed in claim 11.

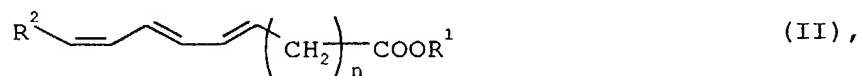
18. A triglyceride with an increased content of saturated fatty acids prepared by a process as claimed in claim 12.

20. (amended) A method for isolating a genomic sequence comprising homology screening with the nucleic acid sequence as claimed in claim 1 or a fragment thereof.

22. An enzyme which converts a fatty acid of the structure I,



which has two double bonds separated from each other by a methylene group, to give a triunsaturated fatty acid of the structure II,



the three double bonds of the fatty acid being conjugated and the substituents and variables in the compounds of the structures I and

MARKED-UP VERSION OF AMENDED CLAIMS

Cancel claims 19 and 21.

5. (amended) A vector comprising a nucleic acid sequence as claimed in claim 1 or a nucleic acid construct [as claimed in claim 4] comprising said nucleic acid sequence linked to one or more regulatory signals.

6. (amended) An organism comprising at least one nucleic acid sequence as claimed in claim 1 or at least one nucleic acid construct [as claimed in claim 4] comprising said nucleic acid sequence linked to one or more regulatory signals.

8. (amended) A transgenic plant comprising a functional or nonfunctional nucleic acid sequence as claimed in claim 1 or a functional or nonfunctional nucleic acid construct [as claimed in claim 4] comprising said nucleic acid sequence linked to one or more regulatory signals.

9. (amended) A process for the preparation of unsaturated fatty acids, which comprises introducing at least one nucleic acid sequence as claimed in claim 1 or at least one nucleic acid construct [as claimed in claim 4] comprising said nucleic acid sequence linked to one or more regulatory signals into an oil-producing organism, growing this organism, isolating the oil contained in the organism and liberating the fatty acids contained in the oil.

10. (amended) A process for the preparation of triglycerides with an increased content of unsaturated fatty acids, which comprises introducing at least one nucleic acid sequence as claimed in claim 1 or at least one nucleic acid construct [as claimed in claim 4] comprising said nucleic acid sequence linked to one or more regulatory signals into an oil-producing organism, growing this organism and isolating the oil contained in the organism.

11. (amended) A process for the preparation of saturated fatty acids, which comprises introducing at least one nonfunctional nucleic acid sequence as claimed in claim 1 or at least one nonfunctional nucleic acid construct [as claimed in claim 4] comprising said nucleic acid sequence linked to one or more regulatory signals into an oil-producing organism, growing this organism, isolating the oil contained in the organism and liberating the fatty acids contained in the oil.

12. (amended) A process for the preparation of triglycerides with an increased content of saturated fatty acids, which comprises introducing at least one nonfunctional nucleic acid sequence as claimed in claim 1 or at least one nonfunctional nucleic acid construct [as claimed in claim 4] comprising said nucleic acid sequence linked to one or more regulatory signals into an oil-producing organism, growing this organism and isolating the oil contained in the organism.

13. (amended) A process as claimed in claim 9 [or 10], wherein the unsaturated fatty acids have an increased calendulic acid content.

14. (amended) A method as claimed in [any of claims 9 to 12] claim 9, wherein the organism is a plant or a microorganism.

20. (amended) [The use of a nucleic acid sequence as claimed in claim 1 or of a fragment thereof] A method for isolating a genomic sequence [via] comprising homology screening with the nucleic acid sequence as claimed in claim 1 or a fragment thereof.

FEUSSNER et al. et al., OZ 0050/50669

REMARKS

The claims have been amended to eliminate multiple dependency and to place them in better form for U.S. practice. Favorable action on the application is solicited.

Respectfully submitted,

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Fatty acid desaturase gene from plants

The present invention relates to a process for the preparation of
5 unsaturated or saturated fatty acids and a process for the
preparation of triglycerides with an increased content of
unsaturated or saturated fatty acids.

Moreover, the invention relates to a nucleic acid sequence; a
10 nucleic acid construct, a vector and organisms comprising at
least one nucleic acid sequence or one nucleic acid construct.
Furthermore, the invention relates to saturated or unsaturated
fatty acids and triglycerides with an increased content of
unsaturated or saturated fatty acids and their use.

15

Fatty acids and triglycerides have a multiplicity of applications
in the food industry, animal nutrition, cosmetics and in the
pharmaceutical sector. Depending on whether they are free
saturated or unsaturated fatty acids or triglycerides with an
20 increased content of saturated or unsaturated fatty acids, they
are suitable for a very wide range of applications; thus, for
example, polyunsaturated fatty acids are added to baby formula to
increase the nutritional value. The various fatty acids and
triglycerides are obtained mainly from microorganisms such as
25 Mortierella or from oil-producing plants such as soya, oilseed
rape, sunflowers and others, where they are usually obtained in
the form of their triacyl glycerides. Alternatively, they are
obtained advantageously from animals, such as fish. The free
fatty acids are prepared advantageously by hydrolysis.

30

Whether oils with unsaturated or with saturated fatty acids are
preferred depends on the intended purpose; thus, for example,
lipids with unsaturated fatty acids, specifically polyunsaturated
fatty acids, are preferred in human nutrition since they have a
35 positive effect on the cholesterol level in the blood and thus on
the possibility of heart disease. They are used in a variety of
dietetic foodstuffs or medicaments.

Especially valuable and sought-after unsaturated fatty acids are
40 the so-called conjugated unsaturated fatty acids, such as
conjugated linoleic acid. A series of positive effects have been
found for conjugated fatty acids; thus, the administration of
conjugated linoleic acid reduces body fat in humans and animals,
and increases the conversion of feed into body weight in the case
45 of animals (WO 94/16690, WO 96/06605, WO 97/46230, WO 97/46118).
By administering conjugated linoleic acid, it is also possible to
positively affect, for example, allergies (WO 97/32008) or cancer

(Banni et al., Carcinogenesis, Vol. 20, 1999: 1019 - 1024, Thompson et al., Cancer, Res., Vol. 57, 1997: 5067 - 5072).

- The chemical preparation of conjugated fatty acids, for example
5 calendulic acid or conjugated linoleic acid, is described in
US 3,356,699 and US 4,164,505. Calendulic acid occurs naturally
in *Calendula officinalis* (Ul'chenko et al., Chemistry of Natural
Compounds, 34, 1998: 272 - 274). Conjugated linoleic acid is
found, for example, in beef (Chin et al., Journal of Food
10 Composition and Analysis, 5, 1992: 185 - 197). Biochemical
studies into the synthesis of calendulic acid can be found in
Crombie et al., J. Chem. Soc. Chem. Commun., 15, 1984: 953 - 955
and J. Chem. Soc. Perkin Trans., 1, 1985: 2425 - 2434.
- 15 Owing to their positive properties, there has been no lack of
attempts in the past to make available genes which participate in
the fatty acid or triglyceride synthesis for the production, in
various organisms, of oils with an altered content of unsaturated
fatty acids. Thus, WO 91/13972 and its US equivalent describe a
20 Δ -9-desaturase. WO 93/11245 claims a Δ -15-desaturase, WO 94/11516
a Δ -12-desaturase. Δ -6-Desaturases are described in WO 93/06712
and WO 96/21022. Other desaturases are described, for example, in
EP-A-0 550 162, WO 94/18337, WO 97/30582, WO 97/21340, WO
95/18222, EP-A-0 794 250, Stukey et al., J. Biol. Chem., 265,
1990: 20144 - 20149, Wada et al., Nature 347, 1990: 200-203 or
25 Huang et al., Lipids 34, 1999: 649 - 659. However, the
biochemical characterization of the various desaturases is as yet
only insufficient since the enzymes, being the membrane-bound
proteins, can only be isolated and characterized with great
difficulty (McKeon et al., Methods in Enzymol. 71, 1981: 12141 -
30 12147, Wang et al., Plant Physiol. Biochem., 26, 1988: 777 -
792).

- In yeasts, both a shift of the fatty acid spectrum toward
unsaturated fatty acids and an increase in productivity were
35 found (see Huang et al., Lipids 34, 1999: 649 - 659, Napier et
al., Biochem. J., Vol. 330, 1998: 611 - 614). However, the
expression of the various desaturases in transgenic plants did
not show the desired success. While it was possible to
demonstrate a shift of the fatty acid spectrum toward unsaturated
40 fatty acids, it emerged, simultaneously, that the synthetic
productivity of the transgenic plants suffered greatly, viz.
lesser amounts of oils were isolated compared with the starting
plants.

- 45 Thus, there remains a great need for new genes which encode
enzymes which participate in the biosynthesis of unsaturated
fatty acids and which allow the latter, specifically conjugated

unsaturated fatty acids, to be synthesized and produced on an industrial scale.

It is an object of the present invention to provide other
5 desaturases for the synthesis of unsaturated conjugated fatty acids.

We have found that this object is achieved by an isolated nucleic acid sequence which encodes a polypeptide with desaturase
10 activity, selected from the following group:

- a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 1,
- 15 b) nucleic acid sequences which, as a result of the degeneracy of the genetic code, are derived from the nucleic acid sequence shown in SEQ ID NO: 1,
- c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 1 which encode polypeptides with the amino acid sequences
20 shown in SEQ ID NO: 2 and which have at least 75% homology at amino acid level without substantially reducing the enzymatic activity of the polypeptides.

25 A derivative (or derivatives) is/are to be understood as meaning, for example, functional homologs of the enzyme encoded by SEQ ID NO: 1 or its enzymatic activity, viz. enzymes which catalyze the same enzymatic reactions as the enzyme encoded by SEQ ID NO:1. These genes also allow an advantageous preparation of unsaturated
30 conjugated fatty acids. Unsaturated fatty acids are to be understood, in the following text, as meaning mono- and polyunsaturated fatty acids whose double bonds may be conjugated or not conjugated. The sequence given in SEQ ID NO:1 encodes a novel, unknown desaturase which participates in the synthesis of
35 calendulic acid in *Calendula officinalis*. The enzyme converts (9Z,12Z)octadecadienoic/linoleic acid to (8E,10E,12Z) octadecaconjugatrienoic/calendulic acid. This is termed calendulic acid desaturase hereinbelow.

40 The nucleic acid sequence according to the invention or its fragments can be used advantageously for isolating further genomic sequences by means of homology screening.

The abovementioned derivatives can be isolated, for example, from other eukaryotic organisms such as plants like *Calendula stellata*, *Osteospermum spinescens* or *Osteospermum hyoseroides*, algae, protozoans such as dinoflagellates, or fungi.

5

Derivatives or functional derivatives of the sequence given in SEQ ID No.1 are furthermore to be understood as meaning, for example, allelic variants which have at least 75% homology at the derived amino acid level, preferably at least 80% homology,

- 10 especially preferably at least 85% homology, very especially preferably 90% homology. The homology was calculated over the entire amino acid range. The program used was PileUp (J. Mol. Evolution., 25 (1987), 351-360, Higgins et al., CABIOS, 5 1989: 151 - 153). The amino acid sequence derived from the
- 15 abovementioned nucleic acid can be seen from the sequence SEQ ID No.2. Allelic variants encompass, in particular, functional variants which can be obtained from the sequence shown in SEQ ID No.1 by means of deletion, insertion or substitution of nucleotides, the enzymatic activity of the derived synthetic
- 20 proteins being retained.

- Such DNA sequences can be isolated from other eukaryotes as mentioned above, starting from the DNA sequence described in SEQ ID No. 1 or parts of these sequences, for example using customary
- 25 hybridization methods or the PCR technique. These DNA sequences hybridize with the sequences mentioned under standard conditions. It is advantageous to use, for the hybridization, short oligonucleotides, for example from the conserved regions, which can be determined by the skilled worker by comparison with other
- 30 desaturase genes.

- Alternatively, it is possible to use longer fragments of the nucleic acids according to the invention or the full sequences for the hybridization. Depending on which nucleic acid:
- 35 oligonucleotide, longer fragment or full sequence, or depending on which nucleic acid type, viz. DNA or RNA, is used for the hybridization, these standard conditions vary. Thus, for example, the melt temperatures for DNA:DNA hybrids are approximately 10°C lower than those of equally long DNA:RNA hybrids.

- 40 Depending on the nucleic acid, standard conditions are understood as meaning, for example, temperatures between 42 and 58°C in an aqueous buffer solution with a concentration of between 0.1 and 5 x SSC (1 x SSC = 0.15 M NaCl, 15 mM sodium citrate, pH 7.2) or additionally in the presence of 50% formamide such as, for
- 45 example, 42°C in 5 x SSC, 50% formamide. The hybridization conditions for DNA:DNA hybrids are advantageously 0.1 x SSC and temperatures between approximately 20°C and 45°C, preferably

5

between approximately 30°C and 45°C. The hybridization conditions for DNA:RNA hybrids are advantageously 0.1 x SSC and temperatures between approximately 30°C and 55°C, preferably between approximately 45°C and 55°C. These temperatures which are indicated for the hybridization are examples of calculated melting point data for a nucleic acid with a length of approx. 100 nucleotides and a G + C content of 50% in the absence of formamide. The experimental conditions for the DNA hybridization are described in relevant genetics textbooks such as, for example, by Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989 and can be calculated using formulae known to the skilled worker, for example as a function of the length of the nucleic acids, the type of hybrid or the G + C content. The skilled worker can find further information on hybridization in the following textbooks: Ausubel et al. (eds), 1985, Current Protocols in Molecular Biology, John Wiley & Sons, New York; Hames and Higgins (eds), 1985, Nucleic Acids Hybridization: A Practical Approach, IRL Press at Oxford University Press, Oxford; Brown (ed), 1991, Essential Molecular Biology: A Practical Approach, IRL Press at Oxford University Press, Oxford.

Derivatives are furthermore to be understood as meaning homologs of the sequence SEQ ID No.1, for example eukaryotic homologs, truncated sequences, simplex DNA of the coding and noncoding DNA sequence or RNA of the coding and noncoding DNA sequence.

Homologs of the sequence SEQ ID No.1 are also to be understood as meaning derivatives such as, for example, promoter variants. These variants can be altered by one or more nucleotide exchanges, by insertion(s) and/or deletion(s), without, however, adversely affecting the functionality or efficacy of the promoters. Moreover, it is possible to increase the efficacy of the promoters by altering their sequence or to exchange them completely by more efficient promoters from other organisms, including other species.

Derivatives are also advantageously to be understood as meaning variants whose nucleotide sequence in the region -1 to -2000 upstream of the start codon was altered in such a way that gene expression and/or protein expression is altered, preferably increased. Moreover, derivatives are also to be understood as meaning variants whose 3' end was altered.

To achieve optimal expression of heterologous genes in organisms, it is advantageous to alter the nucleic acid sequences in accordance with the specific codon usage used in the organism.

The codon usage can be determined readily by using computer evaluations of other, known genes of the organism in question.

- The calendulic acid desaturase gene can be combined
- 5 advantageously in the process according to the invention with other fatty acid biosynthesis genes.

- The amino acid sequences according to the invention are to be understood as meaning proteins which contain an amino acid
- 10 sequence shown in SEQ ID NO: 2 or a sequence obtainable therefrom by the substitution, inversion, insertion or deletion of one or more amino acid residues, the enzymatic activity of the protein shown in SEQ ID NO: 2 being retained or not reduced substantially. The term not reduced substantially is to be
- 15 understood as meaning all enzymes which still have at least 10%, preferably 20%, especially preferably 30% of the enzymatic activity of the starting enzyme. For example, certain amino acids may be replaced by others with similar physico-chemical properties (spatial dimension, basicity, hydrophobicity and the
- 20 like). For example, arginine residues are exchanged for lysine residues, valine residues for isoleucine residues or aspartic acid residues for glutamic acid residues. Alternatively, it is possible to exchange the sequence of, add or remove one or more amino acids, or two or more of these measures may be combined
- 25 with each other.

- The nucleic acid construct or nucleic acid fragment according to the invention is to be understood as meaning the sequence given in SEQ ID NO: 1, sequences which are the result of the genetic
- 30 code and/or their functional or nonfunctional derivatives, all of which have been linked functionally to one or more regulatory signals, advantageously for increasing gene expression. These regulatory sequences are, for example, sequences to which inductors or repressors bind and thus regulate the expression of
- 35 the nucleic acid. In addition to these novel regulatory sequences, or instead of these sequences, the natural regulation of these sequences upstream of the actual structural genes may still be present and, if desired, may have been genetically altered in such a way that the natural regulation has been
- 40 switched off and the expression of the genes increased. However, the expression of the gene construct may also have a simpler structure, viz. no additional regulatory signals have been inserted upstream of the sequence or its derivatives and the natural promoter with its regulation has not been removed.
- 45 Instead, the natural regulatory sequence has been mutated in such a way that regulation no longer takes place and gene expression is increased. These altered promoters may also be placed upstream of the natural gene on their own, in order to increase activity.

- In addition, the gene construct can also advantageously contain one or more so-called enhancer sequences functionally linked to the promoter, and these allow an increased expression of the nucleic acid sequence. It is also possible to insert, at the 3' end of the DNA sequences, additional advantageous sequences such as further regulatory elements or terminators. One or more copies of the calendulic acid desaturase gene may be contained in the gene construct.
- 10 Advantageous regulatory sequences for the process according to the invention are contained, for example, in promoters such as cos, tac, trp, tet, trp-tet, lpp, lac, lpp-lac, lacI^q, T7, T5, T3, gal, trc, ara, SP6, λ -P_R or in the λ -P_L promoter, all of which are advantageously used in Gram-negative bacteria. Other
- 15 advantageous regulatory sequences are contained, for example, in the Gram-positive promoters amy and SPO2, in the yeast or fungal promoters ADC1, MF α , AC, P-60, CYC1, GAPDH, TEF, rp28, ADH, or in the plant promoters such as CaMV/35S [Franck et al., Cell 21(1980) 285-294], PRP1 [Ward et al., Plant.Mol. Biol.22(1993)], SSU, OCS, lib4, STLS1, B33, nos or in the Ubiquitin promoter.
- 20 Other advantageous plant promoters are, for example, a benzenesulfonamide-inducible (EP 388186), a tetracyclin-inducible (Gatz et al., (1992) Plant J. 2,397-404), an abscisic-acid-inducible (EP335528) and an ethanol- or cyclohexanone-inducible (WO9321334) promoter. Other plant
- 25 promoters are, for example, the potato cytosolic FBPase promoter, the potato ST-LSI promoter (Stockhaus et al., EMBO J. 8 (1989) 2445-245), the Glycine max phosphoribosyl pyrophosphate amido transferase promoter (see also gene bank accession number U87999) or a node-specific promoter as described in EP 249676.
- 30 Advantageous plant promoters are, in particular, those which ensure expression in tissues or parts of the plants in which the biosynthesis of fats or their precursors takes place. Promoters which must be mentioned in particular are those which ensure seed-specific expression such as, for example, the USP promoter,
- 35 the LEB4 promoter, the phaseolin promoter or the napin promoter.

In principle, all natural promoters with their regulatory sequences as those mentioned above may be used for the process according to the invention. In addition, synthetic promoters may

40 also advantageously be used.

The nucleic acid fragment (= gene construct, nucleic acid construct) may also contain further genes to be introduced into organisms, as this has been described above. These genes can be

45 under separate regulation or under the same regulatory region as the desaturase gene according to the invention. These genes are, for example, other biosynthesis genes, advantageously of the

fatty acid and lipid biosynthesis, which allow increased synthesis. Examples which may be mentioned are the genes for $\Delta 15$ -, $\Delta 12$ -, $\Delta 9$ -, $\Delta 6$ -, $\Delta 5$ -desaturase, the various hydroxylases, acetylenase, the acyl-ACP thioesterases, the β -ketoacyl-ACP

5 synthases, the acyltransferases such as diacylglycerol acyltransferase, glycerol-3-phosphate acyltransferase or lysophosphatidic acid acyltransferase or β -ketoacyl-ACP reductases. It is advantageous to use the desaturase genes in the nucleic acid construct, especially the $\Delta 12$ -desaturase gene.

10 For expression in a host organism, for example a microorganism such as fungus or a plant, the nucleic acid fragment is advantageously inserted into a vector such as, for example, a plasmid, a phage or other DNA, which vector allows optimal
15 expression of the genes in the host. Examples of suitable plasmids are, in *E. coli*, pLG338, pACYC184, pBR322, pUC18, pUC19, pKC30, pRep4, pHS1, pHS2, pPLc236, pMBL24, pLG200, pUR290, pIN-III¹¹³-B1, λ gt11 or pBdCI, in *Streptomyces* pIJ101, pIJ364, pIJ702 or pIJ361, in *Bacillus* pUB110, pC194 or pBD214, in *Corynebacterium* pSA77 or pAJ667, in fungi pALS1, pIL2 or pBB116,
20 in yeasts 2 μ M, pAG-1, YEpl6, YEpl3 or pEMBLYe23, or, in plants, pLGV23, pGHLac⁺, pBIN19, pAK2004, pVKH or pDH51, or derivatives of the abovementioned plasmids. The plasmids mentioned represent a small selection of the plasmids which are possible. Other
25 plasmids are well known to the skilled worker and can be found, for example, in the book *Cloning Vectors* (Eds. Pouwels P. H. et al. Elsevier, Amsterdam-New York-Oxford, 1985, ISBN 0 444 904018). Suitable plant vectors are described, inter alia, in "Methods in Plant Molecular Biology and Biotechnology" (CRC Press), Chapter 6/7, pp.71-119.

30 In addition to plasmids, vectors are also to be understood as meaning all the other vectors which are known to the skilled worker, such as, for example, phages, viruses such as SV40, CMV, baculovirus, adenovirus, transposons, IS elements, phasmids,
35 phagemids, cosmids, linear or circular DNA. These vectors can be replicated autonomously in the host organism or replicated chromosomally. Chromosomal replication is preferred.

40 The vector advantageously contains at least one copy of the nucleic acid sequence according to the invention and/or of the nucleic acid fragment according to the invention.

To increase the gene copy number, the nucleic acid sequences or homologous genes can be introduced, for example, into a nucleic
45 acid fragment or into a vector which preferably contains the regulatory gene sequences assigned to the genes in question, or

analogously acting promoter activity. Regulatory sequences which are used in particular are those which increase gene expression.

To express the other genes contained, the nucleic acid fragment
5 advantageously additionally contains 3'- and/or 5'-terminal regulatory sequences to increase expression, these sequences being selected for optimal expression, depending on the host organism chosen and the gene or genes.

10 These regulatory sequences should allow the targeted expression of the genes and protein expression. Depending on the host organism, this may mean, for example, that the gene is expressed and/or overexpressed only after induction, or that it is expressed and/or overexpressed immediately.

15 The regulatory sequences or factors can preferably have a positive effect on, and thus increase, the gene expression of the genes introduced. Thus, strengthening of the regulatory elements can advantageously take place at the transcriptional level by
20 using strong transcription signals such as promoters and/or enhancers. In addition, however, strengthening of translation is also possible, for example by improving mRNA stability.

In a further embodiment of the vector, the gene construct
25 according to the invention can advantageously also be introduced into the organisms in the form of a linear DNA and integrated into the genome of the host organism by means of heterologous or homologous recombination. This linear DNA may consist of a linearized plasmid or only of the nucleic acid fragment as vector
30 or of the nucleic acid sequence according to the invention.

The nucleic acid sequence according to the invention is advantageously cloned into a nucleic acid construct together with at least one reporter gene, and the nucleic acid construct is
35 introduced into the genome. This reporter gene should allow easy detectability via a growth assay, a fluorescence assay, a chemo assay, a bioluminescence assay or a resistance assay, or via a photometric measurement. Examples of reporter genes which may be mentioned are genes for resistance to antibiotics or herbicides,
40 hydrolase genes, fluorescence protein genes, bioluminescence genes, sugar metabolism genes or nucleotide metabolism genes, or biosynthesis genes such as the Ura3 gene, the Ilv2 gene, the luciferase gene, the β -galactosidase gene, the gfp gene, the 2-deoxyglucose-6-phosphate phosphatase gene, the β -glucuronidase
45 gene, the β -lactamase gene, the neomycin phosphotransferase gene, the hygromycin phosphotransferase gene or the BASTA (= gluphosinate) resistance gene. These genes allow the

10

transcriptional activity, and thus gene expression, to be measured and quantified easily. In this way, genome sites which show different productivity can be identified.

- 5 In a further advantageous embodiment, the nucleic acid sequence according to the invention may also be introduced into an organism on its own.

If it is intended to introduce, into the organism, other genes in
10 addition to the nucleic acid sequence according to the invention, all can be introduced into the organism in a single vector with a reporter gene, or each individual gene with a reporter gene per vector, it being possible for the various vectors to be introduced simultaneously or in succession.

- 15 The host organism advantageously contains at least one copy of the nucleic acid according to the invention and/or of the nucleic acid construct according to the invention.

- 20 In principle, the nucleic acid according to the invention, the nucleic acid construct or the vector can be introduced into organisms, for example plants, by all methods known to the skilled worker.

- 25 In the case of microorganisms, the skilled worker can find suitable methods in the textbooks by Sambrook, J. et al. (1989) Molecular cloning: A laboratory manual, Cold Spring Harbor Laboratory Press, by F.M. Ausubel et al. (1994) Current protocols in molecular biology, John Wiley and Sons, by D.M. Glover et al.,

- 30 DNA Cloning Vol.1, (1995), IRL Press (ISBN 019-963476-9), by Kaiser et al. (1994) Methods in Yeast Genetics, Cold Spring Harbor Laboratory Press or by Guthrie et al. Guide to Yeast Genetics and Molecular Biology, Methods in Enzymology, 1994, Academic Press.

- 35 The transfer of foreign genes into the genome of a plant is termed transformation. The described methods for the transformation and regeneration of plants from plant tissues or plant cells are used for transient or stable transformation.

- 40 Suitable methods are protoplast transformation by polyethylene-glycol-induced DNA uptake, the use of a gene gun, electroporation, the incubation of dry embryos in DNA-containing solution, microinjection and the agrobacterium-mediated gene transfer. The methods mentioned are described, for example, in B.

- 45 Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press (1993) 128-143 and by Potrykus, Annu.

Rev. Plant Physiol. Plant Molec. Biol. 42 (1991) 205-225). The construct to be expressed is preferably cloned into a vector which is suitable for transforming *Agrobacterium tumefaciens*, for example pBin19 (Bevan et al., Nucl. Acids Res. 12 (1984) 8711).

- 5 The transformation of plants with *Agrobacterium tumefaciens* is described, for example, by Höfgen and Willmitzer in Nucl. Acid Res. 16 (1988) 9877.

- Agrobacteria which have been transformed with an expression
10 vector according to the invention can also be used in the known manner to transform plants such as test plants like *Arabidopsis* or crop plants, in particular oil-containing crop plants such as soya, peanuts, castor, sunflowers, corn, cotton, flax, oilseed rape, coconut palms, oil palms, safflower (*Carthamus tinctorius*)
15 or cacao, for example by bathing wounded leaves or leaf sections in agrobacterial solution and subsequently culturing them in suitable media.

- The genetically altered plant cells can be regenerated by all
20 methods known to the skilled worker. Suitable methods can be found in the abovementioned publications by S.D. Kung and R. Wu, Potrykus or Höfgen and Willmitzer.

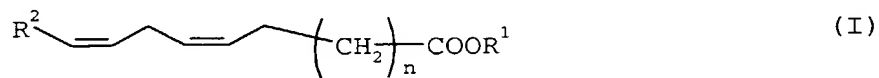
- Suitable organisms or host organisms for the nucleic acid
25 according to the invention, the nucleic acid construct or the vector are, in principle, all organisms which are capable of synthesizing fatty acids, specifically unsaturated fatty acids, and which are suitable for the expression of recombinant genes. Examples which may be mentioned are plants such as *Arabidopsis*,
30 Asteraceae such as *Calendula* or crop plants such as soya, peanuts, castor, sunflowers, corn, cotton, flax, oilseed rape, coconut palms, oil palms, safflower (*Carthamus tinctorius*) or cacao, microorganisms such as fungi, for example the genus *Mortierella*, *Saprolegnia* or *Pythium*, bacteria such as the genus
35 *Escherichia*, yeasts such as the genus *Saccharomyces*, algae or protozoans such as dinoflagellates such as *Cryptothecodinium*. Preferred organisms are those which are naturally capable of synthesizing oils in substantial amounts, like fungi such as *Mortierella alpina*, *Pythium insidiosum* or plants such as soya,
40 oilseed rape, flax, coconut palms, oil palms, safflower, castor, *Calendula*, peanuts, cacao or sunflowers, or yeasts such as *Saccharomyces cerevisiae*, with soya, oilseed rape, flax, sunflowers, *Calendula* or *Saccharomyces cerevisiae* being especially preferred. In principle, transgenic animals, for
45 example *Caenorhabditis elegans*, are also suitable as host organisms.

12

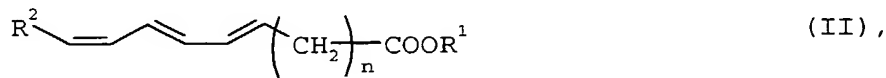
Another embodiment according to the invention are, as described above, transgenic plants which contain a functional or a nonfunctional nucleic acid or a functional or nonfunctional nucleic acid construct. The term nonfunctional is to be understood as meaning that an enzymatically active protein is no longer synthesized since the natural gene has been inactivated. In addition, the term nonfunctional nucleic acids or nucleic acid constructs is also to be understood as meaning a so-called antisense DNA which leads to transgenic plants which show a reduction in, or lack, enzymatic activity. The antisense technology, specifically when combining, in the antisense DNA, the nucleic acid sequence according to the invention with other fatty acid synthesis genes, allows the synthesis of triglycerides with an elevated content of saturated fatty acids, or saturated fatty acids. Transgenic plants are to be understood as meaning single plant cells and their cultures on solid media or in liquid culture, parts of plants and entire plants.

The use of the nucleic acid sequence according to the invention or of the nucleic acid construct according to the invention for the generation of transgenic plants is therefore also subject matter of the invention.

The invention furthermore relates to an enzyme which converts a fatty acid of the structure I,



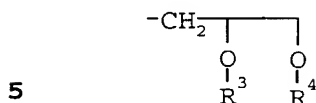
which has two double bonds separated from each other by a methylene group, to give a triunsaturated fatty acid of the structure II,



the three double bonds of the fatty acid being conjugated and the substituents and variables in the compounds of the structures I and II having the following meanings:

13

R¹ = hydrogen, substituted or unsubstituted, unsaturated or saturated, branched or unbranched C₁-C₁₀-alkyl-,



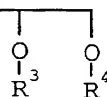
R² = substituted or unsubstituted, unsaturated or saturated C₁-C₉-Alkyl-

10 R³ and R⁴ independently of one another are hydrogen, substituted or unsubstituted, saturated or unsaturated, branched or unbranched C₁-C₂₂-alkylcarbonyl or phospho-,

15 n = 1 to 14, preferably 1 to 8, especially preferably 4 to 6, very especially preferably 6.

R¹ in the compounds of the formula I and II is hydrogen, substituted or unsubstituted, unsaturated or saturated, branched or unbranched C₁-C₁₀-alkyl-, or

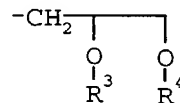
20



Alkyl radicals which may be mentioned are substituted or unsubstituted, branched or unbranched C₁-C₁₀-alkyl chains such as,
 25 for example, methyl, ethyl, n-propyl, 1-methylethyl, n-butyl, 1-methylpropyl-, 2-methylpropyl, 1,1-dimethylethyl, n-pentyl, 1-methylbutyl, 2-methylbutyl, 3-methylbutyl, 2,2-dimethylpropyl, 1-ethylpropyl, n-hexyl, 1,1-dimethylpropyl, 1,2-dimethylpropyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl,
 30 1,1-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl, 3,3-dimethylbutyl, 1-ethylbutyl, 2-ethylbutyl, 1,1,2-trimethylpropyl, 1,2,2-trimethylpropyl, 1-ethyl-1-methylpropyl, 1-ethyl-2-methylpropyl, n-heptyl, n-octyl, n-nonyl or n-decyl.

35

Preferred radicals for R¹ are hydrogen and



40 R² in the compounds of the formula I and II denotes substituted or unsubstituted, unsaturated or saturated C₁-C₉-alkyl-.

Alkyl radicals which may be mentioned are substituted or unsubstituted, branched or unbranched C₁-C₉-alkyl chains such as,
 45 for example, methyl, ethyl, n-propyl, 1-methylethyl, n-butyl, 1-methylpropyl-, 2-methylpropyl, 1,1-dimethylethyl, n-pentyl, 1-methylbutyl, 2-methylbutyl, 3-methylbutyl, 2,2-dimethylpropyl,

- 1-ethylpropyl, n-hexyl, 1,1-dimethylpropyl, 1,2-dimethylpropyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 1,1-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl, 3,3-dimethylbutyl,
- 15 1-ethylbutyl, 2-ethylbutyl, 1,1,2-trimethylpropyl, 1,2,2-trimethylpropyl, 1-ethyl-1-methylpropyl, 1-ethyl-2-methylpropyl, n-heptyl, n-octyl or n-nonyl. C₁-C₅-alkyl is preferred, C₅-alkyl is especially preferred.
- 10 R³ and R⁴ independently of one another are hydrogen, substituted or unsubstituted, saturated or unsaturated, branched or unbranched C₁-C₂₂-alkylcarbonyl- or phospho-.
- C₁-C₂₂-alkylcarbonyl such as methylcarbonyl, ethylcarbonyl,
- 15 n-propylcarbonyl, 1-methylethylcarbonyl, n-butylcarbonyl, 1-methylpropylcarbonyl, 2-methylpropylcarbonyl, 1,1-dimethylethylcarbonyl, n-pentylcarbonyl, 1-methylbutylcarbonyl, 2-methylbutylcarbonyl, 3-methylbutylcarbonyl, 1,1-dimethylpropylcarbonyl,
- 20 1,2-dimethylpropylcarbonyl, 2,2-dimethylpropylcarbonyl, 1-ethylpropylcarbonyl, n-hexylcarbonyl, 1-methylpentylcarbonyl, 2-methylpentylcarbonyl, 3-methylpentylcarbonyl, 4-methylpentylcarbonyl, 1,1-dimethylbutylcarbonyl, 1,2-dimethylbutylcarbonyl, 1,3-dimethylbutylcarbonyl,
- 25 2,2-dimethylbutylcarbonyl, 2,3-dimethylbutylcarbonyl, 3,3-dimethylbutylcarbonyl, 1-ethylbutylcarbonyl, 2-ethylbutylcarbonyl, 1,1,2-trimethylpropylcarbonyl, 1,2,2-trimethylpropylcarbonyl, 1-ethyl-1-methylpropylcarbonyl and 1-ethyl-2-methylpropylcarbonyl, heptylcarbonyl, nonylcarbonyl,
- 30 decylcarbonyl, undecylcarbonyl, n-dodecylcarbonyl, n-tridecylcarbonyl, n-tetradecylcarbonyl, n-pentadecylcarbonyl, n-hexadecylcarbonyl, n-heptadecylcarbonyl, n-octadecylcarbonyl, n-nonadecylcarbonyl or n-eicosylcarbonyl.
- 35 Preferred substituents for R³ and R⁴ are saturated or unsaturated C₁₆-C₂₂-alkylcarbonyl.

Examples of substituents of the abovementioned radicals which may be mentioned are halogen such as fluorine or chlorine, alkyl or

40 hydroxyl.

In the conversion with the enzyme according to the invention, one double bond is introduced into the fatty acid and one double bond is shifted so that the three double bonds which participate in

45 the reaction are conjugated. Furthermore, one double bond is isomerized (from cis to trans).

The enzyme (= calendulic acid desaturase) advantageously catalyzes the conversion of linoleic acid (18:2, 9Z,12Z) to calendulic acid (18:3, 8E,10E,12Z). The enzyme introduces a trans double bond at position C8 and causes the specific shift of a cis double bond in position C9 to a trans double bond in position C10, the isomerization being effected regiospecifically. A possible hypothetical reaction mechanism is shown in Fig. 1. After deprotonation at C8 of the linoleic acid and a rearrangement of the free radical to C10, the elimination of water leads to a deprotonation at C11 and thus to the formation of calendulic acid. Simultaneously, bound FeIV is reduced to FeIII. Fig. 1 shows the hypothetical mechanism for (8,11)-linoleoyl desaturase (calendulic acid desaturase), modified after Svatos, A et al. (Insect Biochemistry and Molecular Biology 29,1999:225-232) based on the proposed catalytic mechanism for Ricinus $\Delta 9$ desaturase (Lindqvist, Y et al., EMBO Journal 15, 1996:4081-4092). Suitable substrates are still 6Z,9Z,12Z, 18:3-fatty acid and 9Z,12Z,15Z, 18:3-fatty acid, which, in turn, are then reacted to give 6Z,8E,10E,12Z- and 8E,10E,12Z,15Z-fatty acids, respectively.

The invention furthermore relates to a process for the preparation of unsaturated fatty acids, which comprises introducing at least one above-described nucleic acid sequence according to the invention or at least one nucleic acid construct according to the invention into a preferentially oil-producing organism, growing this organism, isolating the oil contained in the organism and liberating the fatty acids contained in the oil.

The invention also includes a process for the preparation of triglycerides with an increased content of unsaturated fatty acids, which comprises introducing at least one above-described nucleic acid sequence according to the invention or at least one nucleic acid construct according to the invention into a preferentially oil-producing organism, growing this organism and isolating the oil contained in the organism.

Both processes advantageously allow the synthesis of fatty acids of triglycerides with an increased content of unsaturated fatty acids such as calendulic acid.

The invention furthermore relates to a process for the preparation of saturated fatty acids, which comprises introducing at least one nonfunctional abovementioned nucleic acid sequence according to the invention or at least one nonfunctional nucleic acid construct according to the invention into an oil-producing organism, growing this organism, isolating the oil contained in

the organism and liberating the fatty acids contained in the oil, and to a process for the preparation of triglycerides with an increased content of saturated fatty acids, which comprises introducing at least one nonfunctional abovementioned nucleic acid sequence according to the invention or at least one nonfunctional nucleic acid construct according to the invention into an oil-producing organism, growing this organism and isolating the oil contained in the organism. Both processes involve the use of the so-called antisense technology (see above), or the inactivation of the lateral synthesis genes.

Examples of organisms for the abovementioned processes are plants such as Arabidopsis, soya, peanuts, castor, sunflowers, corn, cotton, flax, oilseed rape, coconut palms, oil palms, safflower (Carthamus tinctorius) or cacao, microorganisms such as the fungi Mortierella, Saprolegnia or Pythium, bacteria such as the genus Escherichia, yeasts such as the genus Saccharomyces, algae or protozoans such as dinoflagellates, for example Cryptocodinium. Preferred organisms are those which can naturally synthesize oils in substantial amounts, such as fungi, for example Mortierella alpina, Pythium insidiosum, or plants such as soya, oilseed rape, flax, coconut palms, oil palms, safflower, castor, Calendula, peanuts, cacao or sunflowers, or yeasts such as Saccharomyces cerevisiae; soya, oilseed rape, flax, sunflowers, Calendula or Saccharomyces cerevisiae are especially preferred.

Depending on the host organism, the organisms used in the processes are grown or cultured in the manner known to those skilled in the art. As a rule, microorganisms are grown in a liquid medium which contains a carbon source, usually in the form of sugars, a nitrogen source, usually in the form of organic nitrogen sources such as yeast extract or salts such as ammonium sulfate, a phosphate source such as potassium hydrogen phosphate, trace elements such as iron salts, manganese salts, magnesium salts and, if required, vitamins, at temperatures between 0°C and 100°C, preferably between 10°C and 60°C, while gassing in oxygen. The pH of the liquid medium can be maintained at a fixed value, i.e. the pH is regulated while culture takes place. However, the microorganisms may also be cultured without pH regulation. Culturing can be effected by the batch method, the semi-batch method or continuously. Nutrients may be supplied at the beginning of the fermentation or fed in semicontinuously or continuously.

Post-transformation, plants are first regenerated as described above and then grown or planted as usual.

17

After the organisms have been grown, the lipids are obtained in the usual manner. To this end, the organisms can first be harvested and then disrupted, or they can be used directly. It is advantageous to extract the lipids with suitable solvents such as

5 apolar solvents, for example hexane, or polar solvents, for example ethanol, isopropanol, or mixtures such as hexane/isopropanol, phenol/chloroform/isoamyl alcohol, at temperatures between 0°C and 80°C, preferably between 20°C and 50°C. As a rule, the biomass is extracted with an excess of

10 solvent, for example with an excess of solvent to biomass of 1:4. The solvent is subsequently removed, for example by distillation. The extraction may also be carried out with supercritical CO₂. After the extraction, the remainder of the biomass can be removed, for example, by filtration. Standard methods for the

15 extraction of fatty acids from plants and microorganisms are described in Bligh et al. (Can. J. Biochem. Physiol. 37, 1959: 911-917) or Vick et al. (Plant Physiol. 69, 1982: 1103-1108).

The crude oil thus obtained can then be purified further, for

20 example by removing cloudiness by adding polar solvents such as acetone or apolar solvents such as chloroform, followed by filtration or centrifugation. Further purification via columns or other techniques is also possible.

25 To obtain the free fatty acids from the triglycerides, the latter are hydrolyzed in the customary manner, for example using NaOH or KOH.

The invention furthermore relates to unsaturated or saturated

30 fatty acids and triglycerides with an increased content of saturated or unsaturated fatty acids which have been prepared by the abovementioned processes, and to their use for the preparation of foodstuffs, animal feed, cosmetics or pharmaceuticals. To this end, they are added to the foodstuffs,

35 animal feed, cosmetics or pharmaceuticals in the customary quantities.

The invention is illustrated in greater detail in the examples which follow:

40

Examples

A cDNA was cloned from *Calendula officinalis* mRNA using RT-PCR and RACE techniques. When expressing this cDNA in yeast, linoleic

45 acid is converted into the octadecaconjugatriene calendulic acid (8E, 10E, 12Z). As far as we know, this is the first time that a calendulic acid desaturase has been described. The enzyme causes

a regiospecific shift of a *cis* double bond in position C9 to a *trans* double bond in position C10 and introduces a new *trans* double bond at position C8.

- 5 Transgenic yeasts and plants with an increased expression of calendulic acid desaturase cDNA show calendulic acid in their lipids.

Example 1: Isolation of RNA from *Calendula officinalis* seeds

10

In order to be able to isolate cDNA clones for calendulic acid desaturase by means of PCR, RNA was isolated from *Calendula officinalis* seeds. Owing to the high fat content of the seeds, it was impossible to use standard protocols; the following method

15 was used instead:

Using a pestle and mortar, 20 g of plant material were ground in liquid nitrogen to give a powder. 100 ml of extraction buffer I [100 mM tris/HCl, pH 7.5, 20 mM EDTA, 2% (w/v) lauryl sarcosyl,
20 4 M guanidinium thiocyanate, 5% (w/v) PVP (= polyvinylpyrrolidone), 1% (v/v) β -mercaptoethanol] were added, and the batch was mixed immediately and homogenized. The solution was transferred into 50-ml-vessels and shaken for approximately 15 minutes. After centrifugation for 10-15 minutes at 4,000 g,
25 the fatty layer or fat drops which had risen to the top were removed and the supernatant was transferred into fresh vessels. This was followed by extraction with 1 volume of phenol/chloroform/isoamyl alcohol (= PCI, 25:24:1) and one extraction with chloroform; in each case, the mixture was shaken
30 for 15 minutes and then centrifuged. The upper, aqueous phase was removed, placed on an 8-ml-CsCl cushion (5 M CsCl) and centrifuged for 18 hours at 18°C and 100,000 g. The supernatant was decanted off and the RNA precipitate was dried briefly. After a washing step with 70% ethanol, the RNA was dissolved in a
35 mixture of 7.5 ml extraction buffer II (100 ml tris/HCl, pH 8.8, 100 mM NaCl, 5 mM EDTA, 2% SDS) and 10 ml of PCI, shaken for 15 minutes and centrifuged. The upper, aqueous phase was extracted with chloroform and then an equal volume of 5 M LiCl was added. The RNA was precipitated overnight at 4°C. The mixture
40 was then centrifuged for 60 minutes at 12,000 g and 4°C. The precipitate was washed twice with 70% ethanol, dried and finally taken up in 500 μ l of H₂O.

mRNA was isolated from the resulting *Calendula* total RNA using
45 the Poly-Attract Kit (Promega, Mannheim) following the manufacturer's instructions. 1 μ g of this mRNA was translated into cDNA with the SuperscriptII reverse transcriptase by Gibco

19

BRL (Eggenstein) using 200 pmol of oligo-dT primer following the manufacturer's instructions and employed as template in a polymerase chain reaction (PCR).

5 Example 2 : Isolation and cloning of the *Calendula officinalis* calendulic acid desaturase

In order to isolate, from *Calendula officinalis*, DNA sequences which encode a calendulic acid desaturase, various degenerate
10 oligonucleotide primers were derived from amino acid sequences of the conserved histidine boxes of various $\Delta 12$ desaturases.

Primer A: 5' - CCD TAY TTC TCI TGG AAR WWH AGY CAY CG - 3'
forward primer, derived from the amino acid sequence
15 P Y F S W K Y/I S H R

Primer B: 5' - CCA RTY CCA YTC IGW BGA RTC RTA RTG - 3'
reverse primer, derived from the amino acid sequence
H Y D S S/T E W D/N W
20

The letters in primers A and B have the following meaning:

R = A/G
Y = C/T
25 W = A/T
H = A/C/T
B = C/G/T
D = A/G/T
I = inositol
30

In a PCR with *Calendula simplex* cDNA (prepared as described in Example 1) as template, a DNA fragment with a length of 470 bp was amplified using primers A and B. The following PCR program was used:

35
1. 2 min 94 °C
2. 30 sec 94 °C
3. 45 sec 50 °C (annealing temperature)
4. 1 min 72 °C
40 10 x 2. to 4.
5. 0 sec 94 °C
6. 45 sec 50 °C
7. 1 min 72 °C, time increment 5 sec per cycle
20 x 5. to 7.
45 8. 2 min 72 °C

20

The Tfi DNA polymerase from Biozym (Hess. Oldendorf) was used for the amplification. The 470 bp DNA fragment was cloned into the vector pCR 2.1-TOPO with the aid of the TOPO TA Cloning Kit (Invitrogen, Carlsbad, USA) and sequenced. The sequence of the
5 470 bp fragment corresponded to the sequence of nucleotide 466 to 893 of SEQ ID NO:1.

Example 3: Obtaining and sequencing complete cDNA clones

- 10 In order to obtain a full-length clone, the fragment was extended by means of 5'- and 3'-RACE (rapid amplification of cDNA ends). Starting from 1 µg of mRNA (isolated as described in Example 1), duplex cDNA was prepared using the "Marathon cDNA Amplification Kit" by CLONTECH (Heidelberg). After ligation of the adaptor, 5'-
15 and 3'-RACE was carried out using the following primers:

Specific primers for 5'-RACE:

- Primer C 5' - GTG AGG GAG TGA GAG ATG GGT GTG GTG C - 3'
20 Primer D 5' - AAC ACA CTT ACA CCT AGT ACT GGA ATT G - 3'

Specific primers for 3'-RACE:

- Primer E 5' - TAT TCC AAA CTT CTT AAC AAT CCA CCC G - 3'
25 Primer F 5'- CAA TTC CAG TAC TAG GTG TAA GTG TGT T - 3'

First, a PCR was carried out with the adaptor-ligated duplex cDNA and primer C or E; then, a second PCR was carried out with primer D or F and a 1:50 dilution of the PCR product from the reaction
30 with primer C or E as template.

The RACE-PCR was carried out using the following program:

- | | | |
|-------|--------------|------|
| 1. | 1 min | 94°C |
| 35 2. | 30 sec | 94°C |
| 3. | 3 min | 68°C |
| | 10 x 2. - 3. | |
| 4. | 30 sec | 94°C |
| 5. | 30 sec | 65°C |
| 40 6. | 3 min | 68°C |
| | 25 x 4. - 6. | |
| 7. | 5 min | 68°C |

The resulting DNA fragments were cloned into pCR 2.1-TOPO with
45 the aid of the TOPO TA Cloning Kit (Invitrogen, Carlsbad, USA) and sequenced. The 5'-RACE product extended over the start codon

21

into the 5'-untranslated region (5'-UTR), and the 3'-RACE over the stop codon into the 3'-UTR).

The composite sequence composed of the first PCR product and the
5 RACE product is shown in SEQ ID NO: 1. The encoding region extends from nucleotide 42 (start codon) to 1175 (stop codon). The 5'- and 3'- UTRs were only sequenced as simplexes, so that individual sequencing mistakes are possible here.

10 In order to obtain an uninterrupted full-length clone, a PCR was carried out using the Expand High Fidelity System (Boehringer, Mannheim) and the primers G and H, with Calendula cDNA (see Example 1) as template.

15 Primer G 5' - ATTAGAGCTCATGGGTGCTGGTGGTCGGATGTCG - 3'
forward primer (with SacI cleavage site)

Primer H 5' - ATTACTCGAGTGACATACACCTTTTGGATTACATCTTG - 3'
reverse primer (with XhoI cleavage site)

20

The PCR was carried out using the following program:

1. 2 min 94°C
2. 30 sec 94°C
- 25 3. 35 sec 63°C
4. 2 min 72°C
- 10 x 2. - 4.
5. 30 sec 94°C
6. 35 sec 63°C
- 30 7. 2 min 72°C, time increment 5 seconds per cycle
- 15 x 5. - 7
8. 2 min 72 °C.

The 1.2 kb PCR product was cloned into the vector pGEM-T (Promega, Mannheim) and transformed into E. coli DH10B. The
35 insert DNA was sequenced as duplex using a 373 DNA sequencer (Applied Biosystems). To this end, the following sequence-specific primers were used in addition to reverse primer and -21 primer:

40 Primer I: 5' - CGG TCT TCT CGC TGT ATT - 3'

Primer J: 5' - ATT ACC CAA GCT GCC C - 3'

45

22

The complete DNA sequence of calendulic acid desaturase (CalDes) is identical to the section from nucleotide 42 to 1193 of SEQ ID NO:1. The sequence encompasses the encoding region and a short section of the 3'-UTR.

5

A comparison of the derived amino acid sequence of Co-CalDes (SEQ ID NO:2) with annotated protein sequences of the SWISS-PROT and SP-TREMBL databases demonstrated the highest homology to a *Crepis alpina* Δ 12-acetylenase (SP_PL: 081931, 74% identical amino acids), a *Crepis palaestina* Δ 12-epoxygenase (SP_PL: 065771, 73% identical amino acids) and a *Borago officinalis* Δ 12-desaturase (SP_PL: 082729, 62% identical amino acids) over the entire encoding region. The sequence comparisons are shown in Fig. 2. Fig. 2 shows a comparisons of the amino acid sequences of Co-CalDes with *Crepis alpina* Δ 12-acetylenase (Ca-Acetyl), *Crepis palaestina* Δ 12-epoxygenase (Cp-Epoxy) and *Borago officinalis* Δ 12-desaturase (Bo-Des).

20

Example 4: Expression of calendulic acid desaturase in yeast

In a first approach, the encoding region of the cDNA was cloned in a yeast expression vector and expressed in *S. cerevisiae*, in order to demonstrate the functionality of CalDes. The calendulic acid desaturase produced in the yeast was meant to convert added linoleic acid into calendulic acid. The latter, in turn, was to be detected by HPLC in hydrolyzed lipid extracts.

In a second approach, the *A. thaliana* Δ 12-desaturase FAD2 (Kajiwara et al., Appl. Environ. Microbiol., 62, 1996: 4309 - 4313) was expressed in yeast in addition to CalDes, so that the yeast cells endogenously produce linoleic acid which, in turn, can be converted into calendulic acid owing to the activity of CalDes. The calendulic acid, in turn, was to be detected by HPLC.

35

All solid and liquid media for yeast were prepared by protocols of Ausubel et al. (Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1995).

The CalDes cDNA was excised from the vector pGEM-T by restriction digest with SacI/XhoI and cloned into the SacI/XhoI-cut shuttle vector pYES2 (Invitrogen, Carlsbad, USA), and the resulting vector pYES2-CalDes was transformed into *E. coli* XL1 blue. After another plasmid preparation with the aid of the Plasmid Maxi Kit (QIAGEN), pYES2-CalDes was transformed into *S. cerevisiae* INCSv1 (Invitrogen, Carlsbad, USA) with the aid of the polyethylene glycol method (Von Pein M., PhD thesis, Heinrich Heine-

23

Universität Düsseldorf, 1992), where the expression of the CalDes cDNA was under the control of the GAL1 promoter.

In order to be able to express, in the second approach, not only CalDes, but also FAD2, in yeast, the encoding region of the FAD2 gene was first amplified via PCR (protocol see Primers G and H) from *A. thaliana* cDNA with the aid of Tfl polymerase (Biozym). The following primers were used for this purpose:

10 Primer K: 5' - AAACCTCGAGATGGGTGCAGGTGGAAGAATGCCGG - 3'
forward primer (XhoI cleavage site)

Primer L: 5' - AAAAAAGCTTTCATAACTTATTGTTGTACCAGTACACACC - 3'
reverse primer (HindIII cleavage site)

15

The resulting PCR product was subjected to a restriction digest with XhoI/HindIII and then cloned into the XhoI/HindIII-cut yeast expression vector pESC-Leu (Stratagene), where the FAD2 DNA was under the control of the GAL1 promoter.

20

The expression of CalDes in *S. cerevisiae* INCSv1 was carried out using a modification of the procedure of Avery et al. (Appl. Environ. Microbiol., 62, 1996: 3960 - 3966) and Girke et al. (The Plant Journal, 5, 1998: 39 - 48). To prepare a starter culture, 10 ml of YPAD medium were inoculated with a single colony and the culture was incubated for 48 hours at 30°C at 200 rpm. Then, the cell culture was washed in 1 x YPA medium without sugar and centrifuged. The pelleted cells were resuspended in 2 ml of minimal medium without supplements and without sugar. 100 ml of minimal medium (dropout powder, 2% raffinose, 1% Tergitol NP40) in 500-ml-Erlenmeyer flasks were inoculated with 1 ml of this cell suspension and the culture was grown at 30°C and 200 rpm. At an OD₆₀₀ of 0.5, 2% (w/v) of galactose were added and (in the case of the first batch) 0.003% of linoleic acid (3% stock solution in 5% Tergitol NP40). The cells were grown on until the stationary phase had been reached. They were then washed in minimal medium without supplements and stored at -20°C.

Example 5: Lipid extraction and HPLC analysis of the fatty acids from transgenic yeast

The yeast cells were suspended in 30 ml of HIP solution (0.1 mM 2,6-di-tert-butyl-4-methylphenol in hexane: isopropanol (3:2 v/v)), acidified with 150 µl of concentrated HCl and homogenized in an Ultra-Turrax (1 min, 24,000 rpm). The samples were then shaken for 10 minutes at 4°C and centrifuged for 10 minutes at 5,000 g and 4°C. The supernatant was transferred into a fresh

container and made up to 47.5 ml with 0.38 M K_2SO_4 . The samples, in turn, were shaken for 10 minutes at 4°C and centrifuged (see above). The hexane phase was withdrawn and evaporated to dryness under a stream of N_2 . The residue was dissolved in 20 µl of
5 chloroform. For the alkaline hydrolysis of fatty acid esters, 400 µl of methanol and 80 µl of 40% strength (w/v) KOH solution were added and the sample was incubated for 20 minutes at 60°C under argon. The sample was subsequently cooled to room temperature, acidified to pH 3.0 with 35 µl of concentrated HCl
10 and separated by HPLC.

The free fatty acids were separated using an ET 250/4 Nucleosil 120-5 C18-column (Macherey & Nagel). The mobile phase used was methanol:H₂O:glacial acetic acid (85:15:0.1 v/v/v). The separation
15 was carried out at a flow rate of 1 ml/min and 25°C, and the absorption was measured at 268 nm to detect the conjutrienes.

Fig. 3 shows the elution profiles of the lipid extracts from transformed yeast cells following alkaline hydrolysis (Fig. 3B,
20 elution profile of *S. cerevisiae* INCSv1 transformed with *A. thaliana* FAD2 DNA, and C, elution profile of *S. cerevisiae* INCSv1 transformed with *Calendula officinalis* pYES2-CalDes), and the elution profile of a calendulic acid standard (Fig. 3A). Calendulic acid has a retention time of 12 minutes with a strong
25 absorption at 268 nm, which is typical for conjutrienes. The hydrolyzed lipid extracts of yeast cells which were transformed with the blank vector pYES2 and grown with 0.003% of linoleic acid show no fatty acids with a retention time of calendulic acid (not shown). Equally, the hydrolyzed lipid extracts of yeast
30 cells which express the FAD2 gene contain no calendulic acid (Fig. 3B).

In contrast, the HPLC analysis of the extracts of pYES2-CalDes-transformed yeast cells grown with 0.003% of
35 linoleic acid showed a signal with the retention time of calendulic acid (Fig. 3C), which also showed the same absorption spectrum as the standard with a maximum of 268 nm and secondary maxima of 258 and 282 nm (Fig. 4A, standard, and C, elution profile of *S. cerevisiae* INCSv1 transformed with *Calendula*
40 *officinalis* pYES2-CalDes). It was thus demonstrated that the expression of calendulic acid desaturase in yeast results in the biosynthesis of calendulic acid. Calendulic acid from transformed yeast cells was only successfully detected after hydrolysis of the lipids. No calendulic acid was detected in the free fatty
45 acids of these cells, that is to say that, in yeast, calendulic acid is incorporated into lipids. Since yeast contains no

triacylglycerides, it must be assumed that the detected calendulic acid had been bound in the phospholipids of the yeast.

In addition, the lipid extracts of transgenic yeast cells which
5 simultaneously express FAD2 and CalDes also contain calendulic acid (not shown).

Example 6: Expression of calendulic acid desaturase in
Arabidopsis thaliana and Linum usitatissimum

10

The expression of Calendula officinalis calendulic acid desaturase in transgenic plants is advantageous for increasing the calendulic acid content in these plants. To this end, the CalDes cDNA was cloned into binary vectors and transferred into
15 A. thaliana and L. usitatissimum via Agrobacterium-mediated DNA transfer. The expression of the CalDes cDNA was under the control of the constitutive CaMV 35S promoter or the seed-specific USP promoter.

20 The expression vectors used were the vector pBinAR (Höfgen and Willmitzer, Plant Science, 66, 1990: 221 - 230) and the pBinAR derivative pBinAR-USP, in which the CaMV 35S promoter had been exchanged for the V. faba USP promoter. For recloning, the CalDes cDNA had to be excised from the vector pGEM-T. To this end, the
25 latter was first cut with NcoI and filled up with Klenow to provide blunt ends; the insert was subsequently excised with SalI and cloned into the SmaI/SalI-cut vectors pBinAR and pBinAR-USP.

The resulting plasmids pBinAR-CalDes and pBinAR-USP-CalDes were
30 transformed into Agrobacterium tumefaciens (Höfgen and Willmitzer, Nucl. Acids Res., 16, 1988: 9877). A. thaliana was transformed by "floral dip" (Clough and Bent, Plant Journal, 16, 1998: 735 - 743), and L. usitatissimum by coculturing linseed hypocotyl sections with transformed A. tumefaciens cells.

35

The expression of the CalDes gene in transgenic Arabidopsis and Linum plants was studied by Northern Blot analysis. Selected plants were studied for their calendulic acid content in the seed oil.

40

To achieve seed-specific expression of CalDes, it is also possible to use the napin promoter analogously to the USP promoter.

45

We claim:

1. An isolated nucleic acid sequence which encodes a polypeptide
5 with desaturase activity, selected from the following group:
 - a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 1,
 - 10 b) nucleic acid sequences which, as a result of the degeneracy of the genetic code, are derived from the nucleic acid sequence shown in SEQ ID NO: 1,
 - c) derivatives of the nucleic acid sequence shown in SEQ ID
15 NO: 1 which encode polypeptides with the amino acid sequences shown in SEQ ID NO: 2 and which have at least 75% homology at amino acid level without substantially reducing the enzymatic activity of the polypeptides.
- 20 2. An amino acid sequence encoded by a nucleic acid sequence as claimed in claim 1.
3. An amino acid sequence as claimed in claim 2, encoded by the
25 sequence shown in SEQ ID NO: 1.
4. A nucleic acid construct comprising a nucleic acid sequence
as claimed in claim 1, where the nucleic acid sequence is
linked to one or more regulatory signals.
- 30 5. A vector comprising a nucleic acid sequence as claimed in
claim 1 or a nucleic acid construct as claimed in claim 4.
6. An organism comprising at least one nucleic acid sequence as
claimed in claim 1 or at least one nucleic acid construct as
35 claimed in claim 4.
7. An organism as claimed in claim 6, which is a plant, a
microorganism or an animal.
- 40 8. A transgenic plant comprising a functional or nonfunctional
nucleic acid sequence as claimed in claim 1 or a functional
or nonfunctional nucleic acid construct as claimed in
claim 4.
- 45 9. A process for the preparation of unsaturated fatty acids,
which comprises introducing at least one nucleic acid
sequence as claimed in claim 1 or at least one nucleic acid

construct as claimed in claim 4 into an oil-producing organism, growing this organism, isolating the oil contained in the organism and liberating the fatty acids contained in the oil.

5

10. A process for the preparation of triglycerides with an increased content of unsaturated fatty acids, which comprises introducing at least one nucleic acid sequence as claimed in claim 1 or at least one nucleic acid construct as claimed in claim 4 into an oil-producing organism, growing this organism and isolating the oil contained in the organism.

10

11. A process for the preparation of saturated fatty acids, which comprises introducing at least one nonfunctional nucleic acid sequence as claimed in claim 1 or at least one nonfunctional nucleic acid construct as claimed in claim 4 into an oil-producing organism, growing this organism, isolating the oil contained in the organism and liberating the fatty acids contained in the oil.

15

20

12. A process for the preparation of triglycerides with an increased content of saturated fatty acids, which comprises introducing at least one nonfunctional nucleic acid sequence as claimed in claim 1 or at least one nonfunctional nucleic acid construct as claimed in claim 4 into an oil-producing organism, growing this organism and isolating the oil contained in the organism.

25

13. A process as claimed in claim 9 or 10, wherein the unsaturated fatty acids have an increased calendulic acid content.

30

14. A method as claimed in any of claims 9 to 12, wherein the organism is a plant or a microorganism.

35

15. An unsaturated fatty acid prepared by a process as claimed in claim 9.

16. A triglyceride with an increased content of unsaturated fatty acids prepared by a process as claimed in claim 10.

40

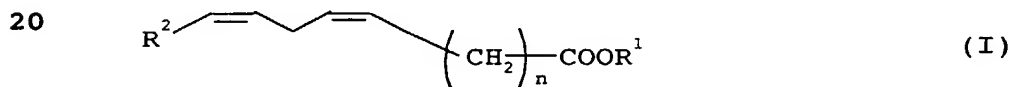
17. A saturated fatty acid prepared by a process as claimed in claim 11.

18. A triglyceride with an increased content of saturated fatty acids prepared by a process as claimed in claim 12.

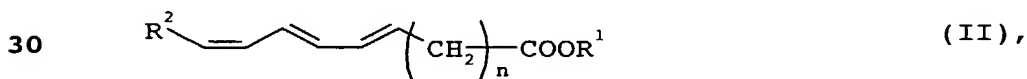
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ART 34 AMDF

19. The use of a nucleic acid sequence as claimed in claim 1 or of a nucleic acid construct as claimed in claim 4 for the generation of transgenic plants.
20. The use of a nucleic acid sequence as claimed in claim 1 or of a fragment thereof for isolating a genomic sequence via homology screening.
21. The use of unsaturated or saturated fatty acids as claimed in claim 15 or 17 or triglycerides with an increased content of unsaturated or saturated fatty acids as claimed in claim 16 or 18 for the preparation of foodstuffs, animal feed, cosmetics or pharmaceuticals.
22. An enzyme which is encoded by a nucleic acid sequence as claimed in claim 1 and which converts a fatty acid of the structure I,



25 which has two double bonds separated from each other by a methylene group, to give a triunsaturated fatty acid of the structure II,



35 the three double bonds of the fatty acid being conjugated and the substituents and variables in the compounds of the structures I and II having the following meanings:

R^1 = hydrogen, substituted or unsubstituted, unsaturated or saturated, branched or unbranched C_1 - C_{10} -alkyl-,



R^2 = substituted or unsubstituted, unsaturated or saturated C_1 - C_9 -Alkyl-

45

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R³ and R⁴ independently of one another are hydrogen,
substituted or unsubstituted, saturated or unsaturated,
branched or unbranched C₁-C₂₂-alkylcarbonyl or phospho-,

5 n = 1 to 14

10

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25

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Fatty acid desaturase gene from plants

Abstract

5

The present invention relates to a process for the preparation of unsaturated or saturated fatty acids and a process for the preparation of triglycerides with an increased content of unsaturated or saturated fatty acids.

10

The invention furthermore relates to a nucleic acid sequence; a nucleic acid construct, a vector and organisms comprising at least one nucleic acid sequence or one nucleic acid construct. Furthermore, the invention relates to saturated or unsaturated

15 fatty acids and triglycerides with an increased content of unsaturated or saturated fatty acids and their use.

20

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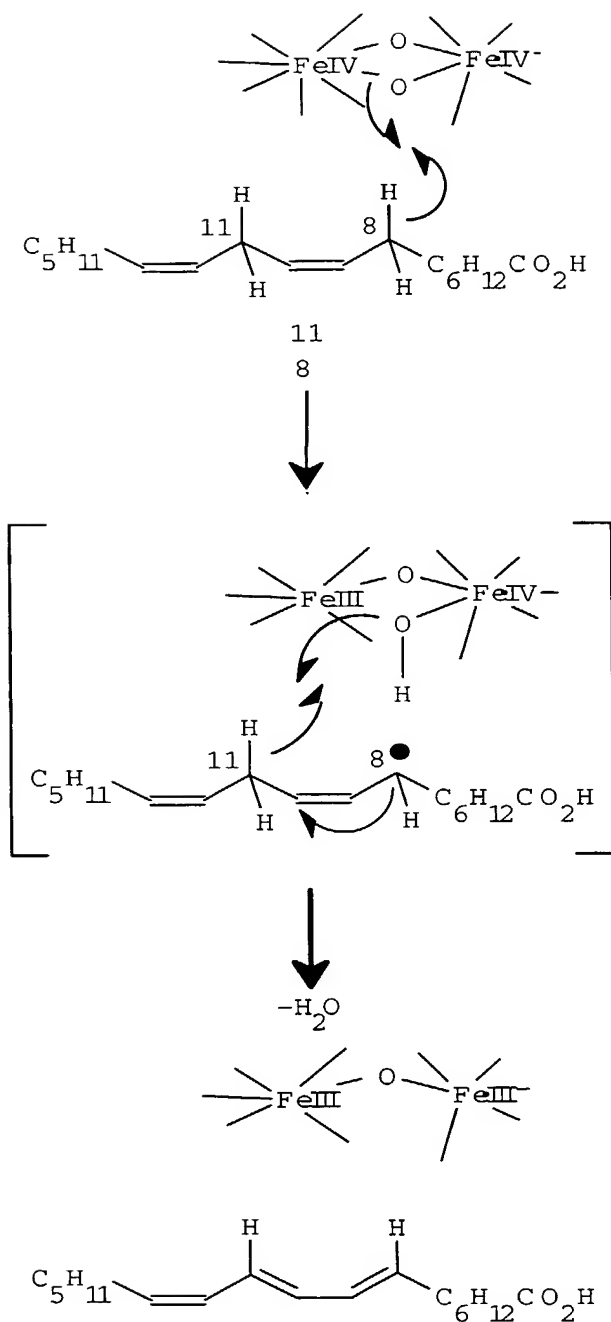
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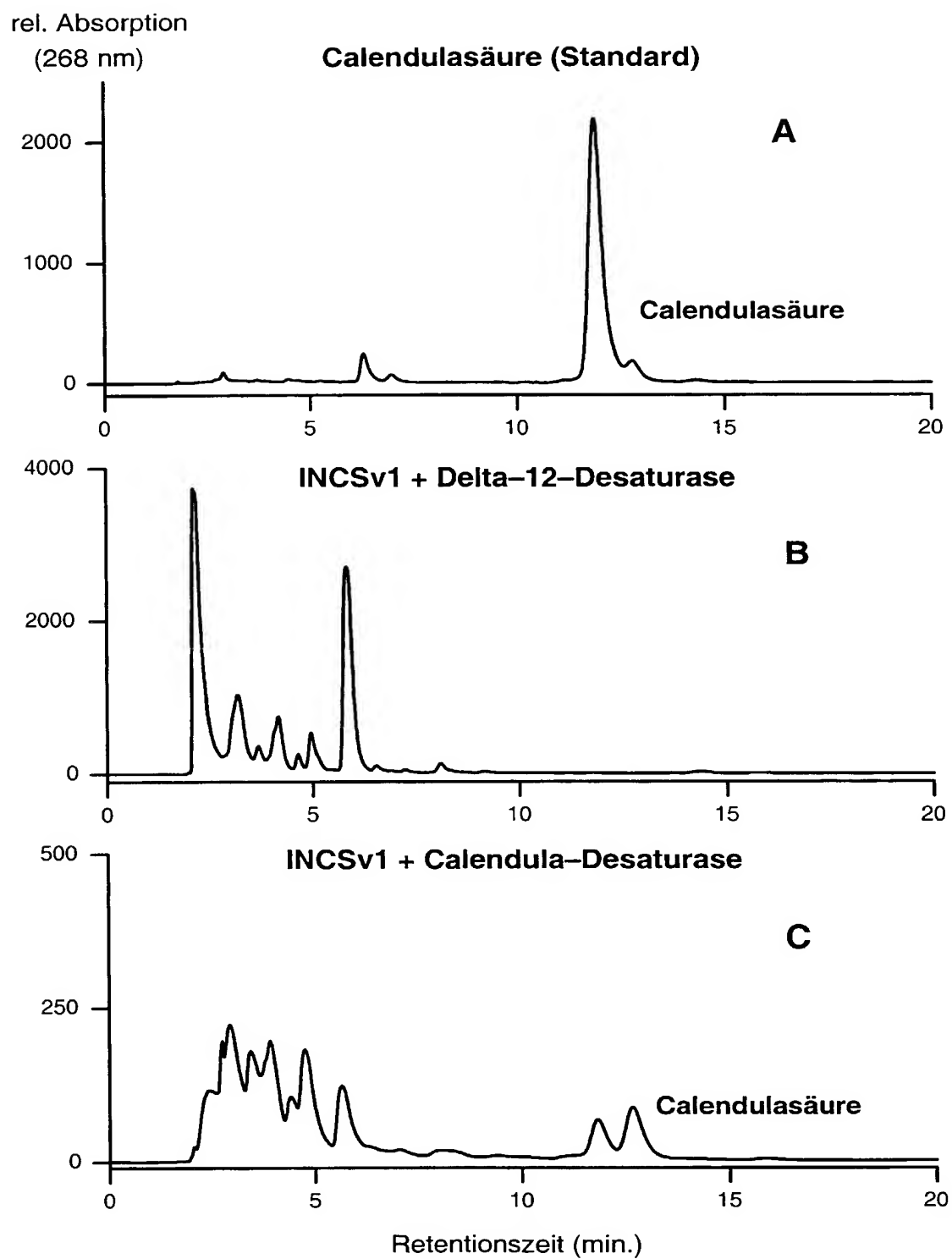
Figur 1



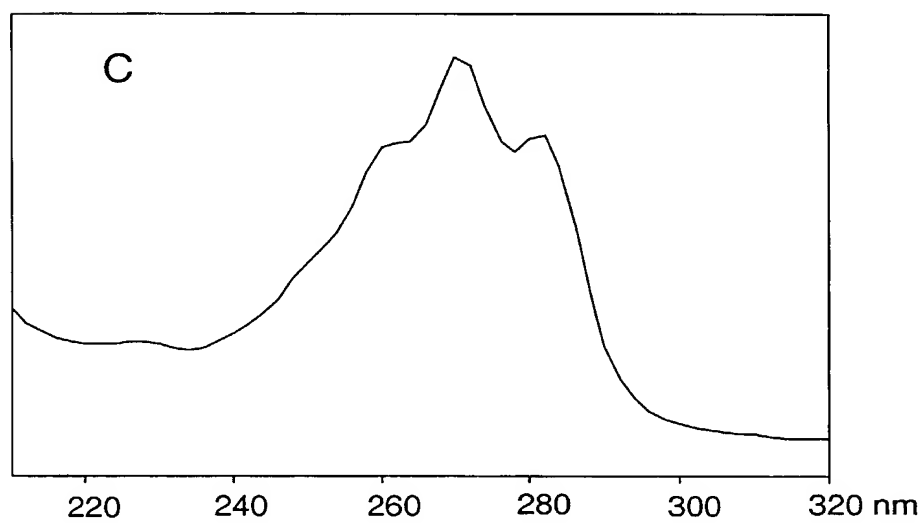
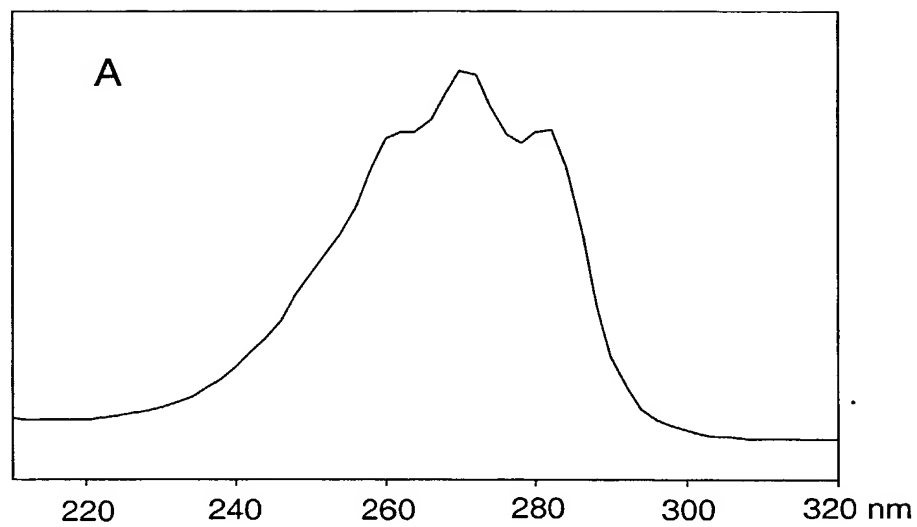
Figur 2

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Co-CalDes	MGAGGRMSDP	SE GK . . . NI	LERVPVDP.P	FTLS DLK KAI	PTHC FERSVI
Ca-Acetyl	MGGGGR.GRT	SQ.K . . . PL	MERVSVD P.P	FTVSDLKQAI	PPHCFKRSVI
Cp-Epoxy	MGAGGR.GRT	SE.K . . . SV	MERVSVD PVT	FSLSELKQAI	PPHCFQRSVI
Bo-Des	MGGGGRMPVP	TKGKSKSDV	FQRPVSEKPP	FTVGD LK KVI	PPHCFQRSVL
	51				100
Co-CalDes	RSSYYVVHDL	IVAYVFYLA	NTYIPLIPTP	LAYLAWPVYW	FCQASILTGL
Ca-Acetyl	RSSYYIVHDA	IIAYIFYFLA	DKYIPILPAP	LAYLAWPLYW	FCQASILTGL
Cp-Epoxy	RSSYYVVQDL	IIAYIFYFLA	NTYIPTLPTS	LAYLAWPVYW	FCQASVLTGL
Bo-Des	HSFSYVVYDL	VIAALFFYTA	SRYIHLQPHP	LSYVAWPLYW	FCQGSVLTGV
	101				150
Co-CalDes	WVIGHECGHH	AFSDYQLIDD	IVGFVLHSAL	LTPYFSWKYS	HRNH HANTNS
Ca-Acetyl	WVIGHECGHH	AFSDYQWVDD	TVGFILHSFL	MTPYFSWKYS	HRNH HANTNS
Cp-Epoxy	WILGHECGHH	AFSNYTWDD	TVGFILHSFL	LTPYFSWKFS	HRNHHSNTSS
Bo-Des	WVIAHECGHH	AFSDYQWLDD	TVGLLLHSAL	LVPYFSWKYS	HRRHHSNTGS
	151				200
Co-CalDes	LDNDEVYIPK	RKSKVKIYSK	LLNNPPGRVF	TLVFRLTLGF	PLYLLTNISG
Ca-Acetyl	LDNDEVYIPK	SKAKVALYK	VLNHPPGRLL	IMFITFTLGF	PLYLFTNISG
Cp-Epoxy	IDNDEVYIPK	SKSKLARIYK	LLNNPPGRLL	VLIIMFTLGF	PLYLLTNISG
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Co-CalDes	KKYGRFANHF	DPMSPIFNDR	ERVQVLLSDF	GLLAVFYAIK	LLVAAKGAAW
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Cp-Epoxy	KKYDRFANHF	DPMSPIFKER	ERFQVFLSDL	GLLAVFYGIK	VAVANKGAAW
Bo-Des	RPYDRFACHF	DPKSPIYNDR	ERLQIYISDA	GIVAVMYGLY	RLVAAKGVAV
	251				300
Co-CalDes	VINMYAIPVL	GVSVFFVLIT	YLHHTHLSLP	HYDSTEWNWI	KGALSTIDRD
Ca-Acetyl	VTCIYGIPVL	GVFIFFDIIT	YLHHTHLSLP	HYDSSEWNWL	RGALSTIDRD
Cp-Epoxy	VACMYGVPVL	GVFTFFDVIT	FLHHTHQSSP	HYDSTEWNWI	RGALSAIDRD
Bo-Des	VVCYYGVPLL	VVNGFLVLIT	YLQHTQPSLP	HYDSSEWDWL	KGALATVDRD
	301				350
Co-CalDes	FGFLNRVFHD	VTHTHVLHHL	ISYIPHYHAK	EARDAIKPVL	GEYYKIDRTP
Ca-Acetyl	FGFLNSVLHD	VTHTHVMHHL	FSYIPHYHAK	EARDAINTVL	GDFYKIDRTP
Cp-Epoxy	FGFLNSVFHD	VTHTHVMHHL	FSYIPHYHAK	EARDAIKPIL	GDFYMIDRTP
Bo-Des	YGFLNKVLHN	ITDTHVAHHL	FSTMPHYHAM	EATKAIKPIL	GDYYQC DRTP
	351			384	
Co-CalDes	IFKAMYREAK	ECIYIEPDED	SEHKG VFWY.	HKM*	
Ca-Acetyl	ILKAMWREAK	ECIFIEPEKG	RESKGVYWY.	NKF*	
Cp-Epoxy	ILKAMWREGR	ECMYIEPDS.	.KLKGVYWY.	HKL*	
Bo-Des	VFKAMYREVK	ECIYVEADEG	DNKKGVFWYK	NKL*	

Figur 3



Figur 4



26

SEQUENCE LISTING

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<130> Sequence_desaturase

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Val Asp Pro Pro Phe Thr Leu Ser Asp Leu Lys Lys Ala Ile Pro Thr
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His Cys Phe Glu Arg Ser Val Ile Arg Ser Ser Tyr Tyr Val Val His
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Asp Leu Ile Val Ala Tyr Val Phe Tyr Tyr Leu Ala Asn Thr Tyr Ile
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cct ctt att cct aca cct ctg gct tac cta gca tgg ccc gtt tac tgg 296
Pro Leu Ile Pro Thr Pro Leu Ala Tyr Leu Ala Trp Pro Val Tyr Trp
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ttt tgt caa gct agc atc ctc acc ggc ctc tgg gtc atc ggt cac gaa 344
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$$x^2 = 0 \quad \text{if } x = 0 \quad \text{and} \quad x^2 = 1 \quad \text{if } x = 1$$
$$x^2 = 0 \quad \text{if } x = 0 \quad \text{and} \quad x^2 = 1 \quad \text{if } x = 1$$
$$x^2 = 0 \quad \text{if } x = 0 \quad \text{and} \quad x^2 = 1 \quad \text{if } x = 1$$

28

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 295 300 305

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 His Val Leu His His Leu Ile Ser Tyr Ile Pro His Tyr His Ala Lys
 310 315 320 325

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 Asp Arg Thr Pro Ile Phe Lys Ala Met Tyr Arg Glu Ala Lys Glu Cys
 345 350 355

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 Tyr Tyr Val Val His Asp Leu Ile Val Ala Tyr Val Phe Tyr Tyr Leu
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 Ala Asn Thr Tyr Ile Pro Leu Ile Pro Thr Pro Leu Ala Tyr Leu Ala
 65 70 75 80

 Trp Pro Val Tyr Trp Phe Cys Gln Ala Ser Ile Leu Thr Gly Leu Trp
 85 90 95

Val	Ile	Gly	His	Glu	Cys	Gly	His	His	Ala	Phe	Ser	Asp	Tyr	Gln	Leu	100	105	110	
Ile	Asp	Asp	Ile	Val	Gly	Phe	Val	Leu	His	Ser	Ala	Leu	Leu	Thr	Pro	115	120	125	
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30

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PTO/PCT Rec'd 10 MAY 2002

HA

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Lys 370	Gly	Val	Phe	Trp	Tyr	His 375	Lys	Met							

Declaration, Power of Attorney and Petition

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Customer No.

26474
PATENT TRADEMARK OFFICE

We (I), the undersigned inventor(s), hereby declare(s) that:

My residence, post office address and citizenship are as stated below next to my name,

We (I) believe that we are (I am) the original, first, and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled

FATTY ACID DESATURASE GENE FROM PLANTS

the specification of which

☐ is attached hereto.

☐ was filed on _____ as

Application Serial No. _____

and amended on _____.

☒ was filed as PCT international application

Number PCT/EP/00/08222

on 23 August 2000,

and was amended under PCT Article 19

on _____ (if applicable).

We (I) hereby state that we (I) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We (I) acknowledge the duty to disclose information known to be material to the patentability of this application as defined in Section 1.56 of Title 37 Code of Federal Regulations.

We (I) hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed. Prior Foreign Application(s)

Application No.	Country	Day/Month/Year	Priority Claimed
19941609.5	Germany	01 September 1999	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

We (I) hereby claim the benefit under Title 35, United States Codes, § 119(c) of any United States provisional application(s) listed below.

(Application Number)

(Filing Date)

(Application Number)

(Filing Date)

We (I) hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial No.

Filing Date

Status (pending, patented,
abandoned)

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And we (I) hereby appoint **Messrs. HERBERT. B. KEIL**, Registration Number 18,967; and **RUSSEL E. WEINKAUF**, Registration Number 18, 495; the address of both being Messrs. Keil & Weinkauf, 1101 Connecticut Ave., N.W., Washington, D.C. 20036 (telephone 202-659-0100), our attorneys, with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to sign the drawings, to receive the patent, and to transact all business in the Patent Office connected therewith.

We (I) declare that all statements made herein of our (my) own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Declaration

Page 4 of 4

0050/050669

5-10

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Signature of Inventor

Date September 7, 2000

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